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CASE REPORT

Occupational Asthma Induced by *Chrysonilia sitophila* in a Worker Exposed to Coffee Grounds▽

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A new case of occupational asthma caused by *Chrysonilia sitophila* (asexual state of *Neurospora sitophila*) was diagnosed by molecular identification of the mold and confirmed by skin prick test, peak expiratory flow rate measurements, and experimental immunoglobulin E analysis.

CASE REPORT

A 43-year-old previously healthy man, who was a nonsmoker, was examined for repetitive episodes of coughing, dyspnea, rhinitis, and conjunctivitis related to his job. He was employed as a coffee dispenser operator for 13 years. His job consisted of emptying containers of coffee grounds and placing new powdered coffee into beverage dispensers. After 9 years of employment, he developed respiratory and ophthalmological symptoms when he collected coffee grounds that had been stored for longer than a week. These coffee grounds were covered with an orange powder, which dispersed into the air when he cleaned the machine. He had no symptoms during weekends and holidays or when the coffee dispenser was emptied frequently and the coffee grounds were not covered by the orange powder.

The patient had no history of rhinitis or asthma. He had no pets at home and stopped smoking 15 years ago. His father and sister have a history of pollen rhinitis.

We performed a mycological analysis of the orange powder covering a coffee grounds sample brought by the patient. Only one type of mold grew quickly and displayed floccose salmon-colored colonies. Microscopic examination showed septate hyphae with lateral branches forming chains of conidia and arthroconidia. These aspects are characteristic of *Chrysonilia sitophila*, as described by de Hoog et al. (2). The last colonies were subcultured successively twice to obtain pure cultures of *C. sitophila*. A fresh coffee sample, brought by the patient was examined for fungal culture and was negative. DNA extraction, amplification, and sequencing of the intergenic transcribed spacer and 5.8S regions of fungal ribosomal genes were performed from the pure culture (8). The nucleotide sequence obtained was deposited in GenBank and aligned and compared with reference sequences present in the database, using Basic Local Alignment Search Tool (BLAST) searches at the National Center for Biotechnology Information (NCBI) web interface (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Molecular diagnosis confirmed the mycological identification.

Skin prick tests (SPTs) were performed with 20 common aeroallergens and included phosphate codeine (positive) and saline (negative) as controls. SPTs were positive for extracts of grass pollen and birch pollen (Stallergènes, France), revealing wheal and flare responses of 7/30 mm and 5/30 mm, respectively. SPTs were negative for mold extracts of *Alternaria* and *Cladosporium*. The SPT with the coffee grounds covered by the orange powder, prepared as a 1/100 (wt/vol) solution and diluted 1/10, was positive and induced a wheal and flare response of 7/25 mm. The SPT with a fresh coffee sample brought by the patient was negative.

Basal spirometry and chest X ray were normal. Nonspecific bronchial challenge with metacholine (100 μg) confirmed the diagnosis of asthma (45% decrease in forced expiratory volume in 1 s [FEV1] reversed by salbutamol). Serial measurements of the peak expiratory flow rate (PEFR) showed an immediate decrease, by more than 20%, when the patient was exposed to the coffee grounds covered by the orange powder during his occupational activity. The patient had normal values during weekends and holidays. The patient provided informed consent before SPTs performed in the medical department and before PEFR measurements during his usual occupational activity.

Specific immunoglobulin E (IgE) measurements were positive for *Betula verrucosa* and *Dactylis glomerata* (birch and grass pollens, respectively), as well as *Alternaria tenuis* (72.8 kU/liter, 7.9 kU/liter, and 0.19 kU/liter, respectively), and negative for a mixture of *Aspergillus* spp., using ImmunoCAP (Phadia, Uppsala, Sweden). Specific IgE measurements for *C. sitophila* pure culture isolated from the coffee grounds were determined by streptavidin ImmunoCAP, a solid phase assay for the coupling of new allergens (6). *C. sitophila* extract was prepared by freeze-drying from the pure culture in Phadia's laboratory in

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Sweden. *C. sitophila* extract (10 mg/ml) was biotinylated for 2 h on a rotator at room temperature with D-biotinoyl-ε-aminocaproic acid *N*-hydroxysuccinimide ester (NHS-biotin) in 10 mM carbonate buffer (pH 8.5). Biotinylated allergen was coupled with prewashed streptavidin ImmunoCAP, incubated for 30 min, washed as previously described (6), and used for the measurement of IgE antibodies in patient serum. The specific IgE value obtained through this process was significant: 11.4 kU/liter. Specific IgE values for coffee and green coffee bean were negative using ImmunoCAP.

The patient was treated with a combination of corticosteroids and bronchodilators through inhalation and antihistaminic drugs. The patient was advised to wear a class 2 respiratory mask (particulate respirator) and to empty the coffee dispensers more frequently, to prevent the growth of *C. sitophila*. The employer was advised to modify the "coffee grounds" evacuation system, to avoid dispersion of the powder into the air.

Molds are aeroallergens, which may induce asthma. The most common allergenic molds are anamorphic fungi like *Alternaria alternata*, *Aspergillus* spp., and *Cladosporium* spp. (4). The genus *Neurospora* is less frequently found to be an aeroallergen among these fungi. However, the role of molds is seldom reported in occupational asthma (OA). New types of occupational exposure can cause sensitivity to molds and the development of OA. Recently, two cases of OA induced by *Neurospora sitophila* (the sexual state of *C. sitophila*) have been published in the coffee industry (3, 5). We report a third case of OA, which consists of a coffee dispenser operator who developed sensitivity to *C. sitophila*, found as an orange powder on the coffee grounds. Other cases of occupational asthma induced by *C. sitophila* in woodcutting (7) and induced by other *Neurospora* species in plywood factories (1) have been previously published. *C. sitophila* is commonly known as the orange bread mold frequently found on foodstuffs, vegetation, and cork and can grow in a hot and humid environment such as in coffee dispensers.

The diagnosis was based on the relationship between respiratory symptoms and occupational exposure to coffee grounds with orange powder. We identified *C. sitophila* and demonstrated that the patient was sensitive to this mold through positive skin prick test results, peak expiratory flow rate (PEFR) decrease, and specific IgE antibody levels. The atopic status of the patient was probably a risk factor in his sensitization and development of allergic occupational asthma. Frequent occupational exposure was probably necessary to induce sensitization and asthma.

The recognition of a new causal occupational allergen is essential for diagnosis, therapy, and prevention. Measurement of specific IgE antibody levels in relation to a new allergen, if no other validated test system is available, requires a valuable method for coupling the new allergen. We used the streptavidin ImmunoCAP assay, a particularly useful tool for detecting IgE antibodies to new allergens in the field of occupational allergies.

C. sitophila should be considered as an allergen for OA in the coffee industry. To our knowledge, this is the first report in which fungal molecular identification of this allergen was performed.

Nucleotide sequence accession number. The nucleotide sequence obtained in this study has been deposited in GenBank under accession no. GU192459.

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We have no potential conflicts of interest.

REFERENCES

1. **Cote, J., H. Chan, G. Brochu, and M. Chan-Yeung.** 1991. Occupational asthma caused by exposure to *Neurospora* in a plywood factory worker. Br. J. Ind. Med. **48:**279–282.
2. **De Hoog, G. S., J. Guarro, J. Gené, and M. J. Figueras.** 2000. Atlas of clinical fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands.
3. **Heffler, E., F. Nebiolo, S. Pizzimenti, M. Ferlini, C. Marchese, and G. Rolla.** 2009. Occupational asthma caused by *Neurospora sitophila* sensitization in a coffee dispenser service operator. Ann. Allergy Asthma Immunol. **102:**168–169.
4. **Horner, W. E., A. Helbling, J. E. Salvaggio, and S. B. Lehrer.** 1995. Fungal allergens. Clin. Microbiol. Rev. **8:**161–179.
5. **Monzon, S., J. Gil, A. Ledesma, L. Ferrer, S. San Juan, and T. Abos.** 2009. Occupational asthma IgE mediated due to *Chrysonilia sitophila* in coffee industry. Allergy **64:**1686–1687.
6. **Sander, I., S. Kespohl, R. Merget, N. Goldscheid, P. O. Degens, T. Brüning, and M. Raulf-Heimsoth.** 2005. A new method to bind allergens for the measurement of specific IgE antibodies. Int. Arch. Allergy Immunol. **136:** 39–44.
7. **Tarlo, S. M., Y. Wai, J. Dolovich, and R. Summerbell.** 1996. Occupational asthma induced by *Chrysonilia sitophila* in the logging industry. J. Allergy Clin. Immunol. **97:**1409–1413.
8. **White, T., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–325. *In* M. A. Innis, D. H. Gelfand J. J. Sninsky, and T. J. White (ed), PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA.